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Distribution of the Beijing Family Genotypes of *Mycobacterium tuberculosis* in Taiwan

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To investigate the distribution of the Beijing family genotypes of *Mycobacterium tuberculosis* in Taiwan, we collected 421 *M. tuberculosis* complex clinical isolates at random from four geographic regions of Taiwan and analyzed them by spacer oligonucleotide typing (spoligotyping) in 2003. We found 113 resolved spoligotypes, among which we identified 28 (24.8%) clusters. One hundred eighty-seven (44.4%) isolates were Beijing family genotypes and consisted of 172 (40.9%) characteristic Beijing genotypes and 15 (3.6%) Beijing-like ones. We also found that substantially larger proportions of tuberculosis patients were infected with Beijing family genotypes in the northern (51.6%) and eastern (46.2%) regions of Taiwan, while 31.6 and 28.0% of the tuberculosis patients in the central and southern regions, respectively, were infected with these genotypes. The proportion of Beijing genotype isolates was the highest in patients below the age of 24 years (61.5%), the second highest in elderly patients over age 65 years (46.8%), and the lowest in middle-age patients between the ages of 45 and 54 years (34.0%). Geographic location and age were found by multivariate analysis to be associated with Beijing family genotypes. Antituberculosis drug resistance was found more often in Beijing family genotype strains (46.4%) than in non-Beijing family genotype strains (34.3%), with more Beijing family genotype strains being resistant to ethambutol and isoniazid. These findings suggest that *M. tuberculosis* Beijing family genotypes have been dominant for several decades and that they are the cause of a significant proportion of the recent transmissions of tuberculosis in Taiwan.

Since Taiwan implemented its National Tuberculosis Program more than 50 years ago, the area has seen significant decreases in the prevalence of and rates of mortality from tuberculosis. Despite these decreases, tuberculosis remains a leading notifiable infectious disease on the island. In 2001, 14,486 cases were reported, for a notification rate of 64.9 per 100,000 people (10).

Spacer oligonucleotide typing (spoligotyping) is a type of molecular genotyping technique used in surveillance studies to track the transmission of infectious diseases caused by many strains, especially Beijing family genotype strains (6, 16, 17, 22). Molecular genotyping methods are being used more and more to track the transmission of infectious diseases. The discovery of genetic markers for *M. tuberculosis* has facilitated the development of large-scale and reproducible fingerprinting methods (18, 35), particularly spoligotyping, which has made feasible the simultaneous detection and differentiation of *M. tuberculosis* strains. Spoligotyping is a PCR-based method capable of analyzing the strain-dependent polymorphisms in the *M. tuberculosis* short direct repeat (DR) chromosomal region, which consists of identical 36-bp DRs interspersed with 35- to 41-bp nonrepetitive spacer sequences.

Beijing family genotypes of *M. tuberculosis* were first recognized in 1995 (31). They are reported to account for 86% of the tuberculosis isolates from Beijing, China (31), and a high proportion of isolates from Mongolia, Thailand, and South Korea (34). However, Beijing family genotype strains are relatively rare in other regions of the world, such as Finland (30) and India (25). It has been reported that Beijing family genotype strains are also common in Hong Kong (9), Malaysia (11), Vietnam (2, 20, 21), and Thailand (29) and might potentially become predominant strains if they are introduced into a new population (8). In Vietnam, for example, strains of the Beijing family genotype have been associated with infections in younger people and are thought to be related to the recent transmission of tuberculosis there. Beijing family genotype strains have also been associated with the transmission of drug-resistant tuberculosis in Germany (28), Cuba (13), Estonia (19), Russia (26, 27, 32), and South Africa (27). In the United States, a highly drug resistant strain, strain W, also belongs to the Beijing family (1, 4, 5). The purpose of this study was to use spoligotyping to investigate the distribution of Beijing family genotype *M. tuberculosis* in Taiwan.

MATERIALS AND METHODS

Study population and bacterial isolates. Isolates were collected in 2002 from the mycobacteriology laboratories of five general hospitals located in four geographical regions in Taiwan, namely, Taipei Veterans General Hospital (northern region), National Taiwan University Hospital (northern region), Buddhist Tzu Chi General Hospital (eastern region), National Cheng-Kung University Hospital (southern region), and Chung-Shan Medical University Hospital (central region). One isolate bacteriologically and biochemically confirmed to be a member of the *M. tuberculosis* complex was collected from each patient. All isolates from new or retreated pulmonary or extrapulmonary tuberculosis patients were included. Demographic data, including the identification number, sex, age, and history of tuberculosis for the patients and the isolate drug resistance patterns and sources, were collected and entered into a computer by using Microsoft Access software. The results of drug susceptibility tests obtained by the

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agar proportion method (National Taiwan University Hospital and Chung-Shan Medical University Hospital), *t* test (Taipei Veterans General Hospital), or Mycobacteria Growth Indicator Tube (Buddhist Tzu Chi General Hospital and National Cheng-Kung University Hospital) were recorded, if they were available. For the agar proportion method, the critical concentrations in the medium were 0.2 and 1.0 μg/ml for isoniazid, 2.0 and 10.0 μg/ml for streptomycin, 5.0 and 10.0 μg/ml for ethambutol, and 1.0 and 5.0 μg/ml for rifampin. An isolate was defined to be drug resistant when the medium with the drug developed more than 1% of the number of tubercle bacillus colonies found in the drug-free medium. Isolates resistant to at least isoniazid and rifampin were considered multidrug-resistant *M. tuberculosis*.

Genotyping method. Spoligotyping was used to identify and differentiate the *M. tuberculosis* complex isolates in this study (17). A commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands) was used as described by the manufacturer. Briefly, the amplified DNA was hybridized to a membrane covalently precoated with a set of 43 spacer oligonucleotides derived from the spacer sequences of *M. tuberculosis* H37Rv and *M. bovis* P3. Both *M. tuberculosis* and *M. bovis* reference strains were included in each test as positive controls. Either chromosomal DNA or a boiled bacterial suspension was subjected to a PCR with primers DRa (5′-CCG AGA GGG GAC GGA AAC-3′) and DRb (5′-GGT TTT GGG TCT GAC GAC-3′). The resulting PCR products were then hybridized with the precoated membrane, and the final image was detected with an enhanced chemiluminescence system. The spoligotypes resolved were *M. tuberculosis* complex strain specific. Only strains that hybridized to all of the last nine spacer oligonucleotides (spacers 35 to 43) were defined as characteristic Beijing genotype strains, whereas strains that hybridized to only some of the last nine spacers were defined as Beijing-like genotype strains. Therefore, the Beijing family strains in this study included all strains with the characteristic Beijing genotypes and strains with possible Beijing-like genotypes. Strains of all other spoligotypes were defined as non-Beijing genotype strains. Strains whose spoligotypes did not have spacers 29 to 32 or 34 but that had spacer 33 were classified as ancestral *M. tuberculosis* strains (26).

Computer-assisted pattern and statistical analysis. The spoligotypes were scanned and analyzed with Bionumerics software (version 2.0; Applied Maths, Kortrijk, Belgium). The software performed automatic pattern identification, confirmed by visual evaluation of the image. A comparison of spoligotype similarity was done by use of the unweighted pair group method with arithmetic averages algorithm, which made use of the clustering of arithmetic averages method and which applied the Dice coefficient, according to the instructions of the manufacturer. A cluster was defined as a group of two or more isolates of the same spoligotypes. Statistical analysis was performed with STATA software (intercooled version 8.0; STATA Corporation, Houston, Tex.) and EpiInfo software (version 6.04; Centers for Disease Control and Prevention, Atlanta, Ga.). Categorical variables were analyzed by Pearson's χ^2 test and multivariate logistic regression analysis.

RESULTS

A total of 421 patients were selected at random from the four geographic regions of Taiwan, and only one isolate from each patient was examined. Patient characteristics are shown in Table 1.

A total of 113 spoligotypes were found among the 421 isolates (Fig. 1). Twenty-eight (24.8%) of the spoliogtypes had clustering patterns (Fig. 2), while the other 85 (75.2%) had unique patterns. One hundred eighty-seven (44.4%) of the isolates belonged to the Beijing family: 172 (40.9%) were characteristic Beijing genotype strains (cluster 3) and 15 (3.6%) were Beijing-like genotype strains (clusters 1, 4, 5, and 28; Fig. 2). The other 243 (55.6%) isolates were non-Beijing genotype strains.

The proportions of patients infected with Beijing family genotype strains, stratified by region, sex, and age group, are shown in Table 2. The percentage of patients with tuberculosis infected with Beijing family genotypes was higher in the northern (51.6%) and eastern (46.2%) regions of Taiwan than in the central (31.6%) and southern (28.0%) regions. The predominant *M. tuberculosis* strains (25 of 75; 33.3%) in the southern region of Taiwan (cluster 23; Fig. 2) were found to have a spoligotyping profile characteristic of that of ancestral strain types instead of that of modern strains.

TABLE 1. Beijing family genotype *M. tuberculosis* complex isolates from 421 tuberculosis cases in 2002 in Taiwan, by region, sex, and age group

Characteristic	No. (%) of isolates	No. (%) of isolates of Beijing family genotype	Univariate analysis	
			Odds ratio	95% CI
Total	421	187 (44.4)		
Region				
Northern	215 (51.1)	111 (51.6)	2.7	1.5–5.1
Central	38 (9.0)	12 (31.6)	1.2	0.5–3.0
Southern	75 (17.8)	21 (28.0)	1.0	
Eastern	93 (22.1)	43 (46.2)	2.2	1.1–4.5
Sex				
Male	322 (76.5)	144 (44.7)	1.1	0.7–1.7
Female	99 (23.5)	43 (43.4)	1.0	
Age group (yr)				
<24	26 (6.2)	16 (61.5)	3.1	1.0–9.5
25–34	35 (8.3)	17 (48.6)	1.8	0.7–5.0
35–44	43 (10.2)	18 (41.9)	1.4	0.5–3.6
45–54	47 (11.2)	16 (34.0)	1.0	
55–64	52 (12.4)	18 (34.6)	1.0	0.4–2.6
>65	218 (51.8)	102 (46.8)	1.7	0.8–3.5

The percentage of men infected with Beijing family genotype strains was about the same as that of women. The prevalence of Beijing genotype strains was higher among patients in the youngest age group (under age 24 years; 61.5%) and among those in the elderly group (over age 65 years; 46.8%) than it was among the rest of the patients. In the northern and central regions of Taiwan, 90% (9 of 10) and 100% (2 of 2) of the isolates obtained from patients under age 24 years were Beijing family genotype strains, respectively. Univariate analysis showed geographic location to be associated with Beijing family genotypes (Table 1). Multivariate analysis found statistically significant associations between the Beijing family genotype strains and residence in the northern region (95% confidence interval [CI], 1.7 to 5.3), residence in the eastern region (95% CI, 1.2 to 4.7), and age less than 24 years (95% CI, 1.3 to 10.1).

Drug susceptibility testing results were available for only 353 of the 421 isolates collected for this study. The drug resistance profiles for the Beijing family and the non-Beijing family strains are shown in Table 3. The rates of antituberculosis drug resistance, especially the rates of resistance to isoniazid (33.7%; 95% CI, 1.0 to 2.6%) and ethambutol (27.0%; 95% CI, 1.1 to 3.2%), were significantly higher among the Beijing family strains than among the non-Beijing family strains.

DISCUSSION

Studies with an immunocompetent mouse model have reported that some Beijing family strains are hypervirulent (24) and that they are more likely to be resistant to *M. bovis* BCG vaccination (23). However, research on the characteristics and epidemiology of the Beijing family genotype strains is limited (22). Some epidemiological studies have investigated the prev-

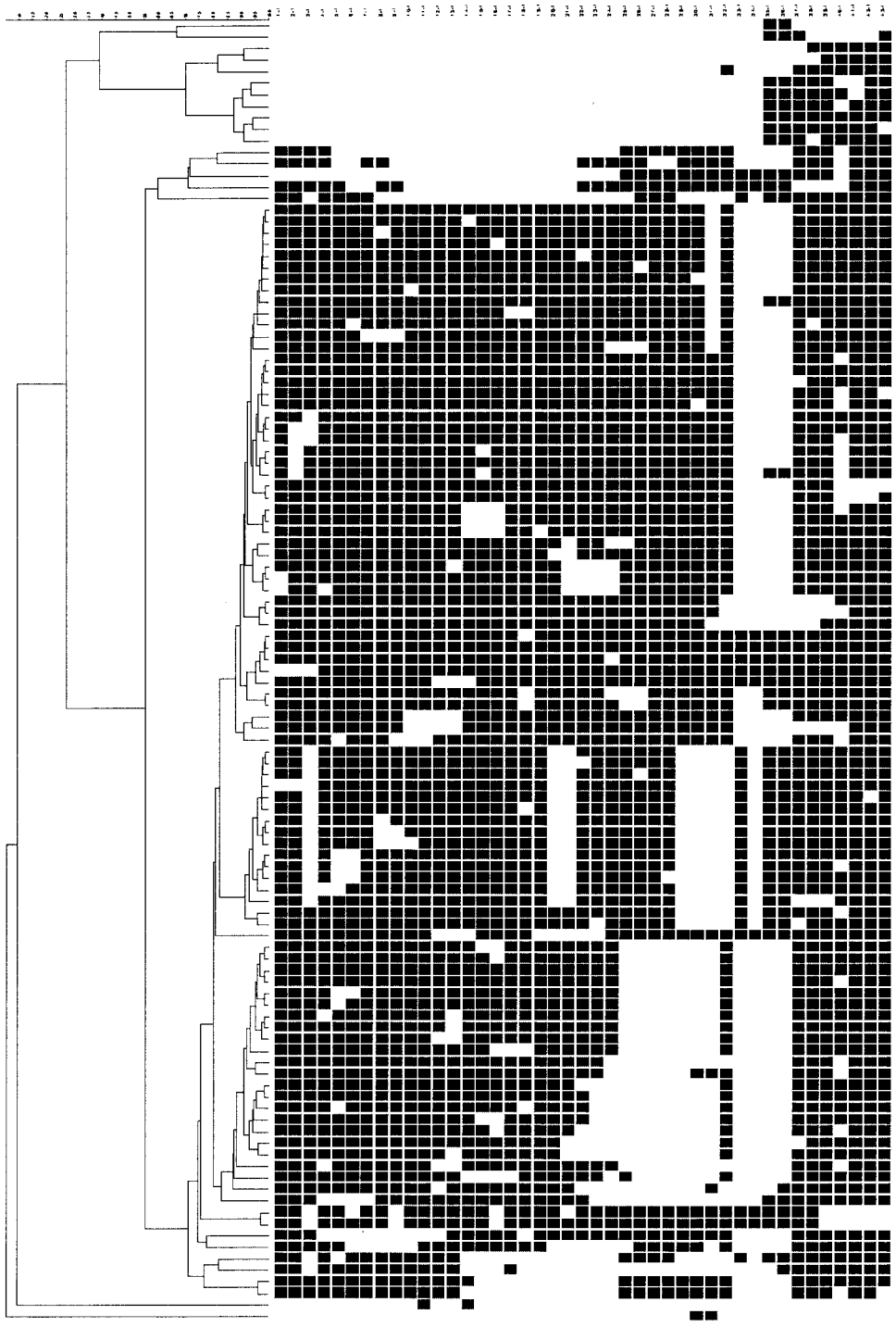
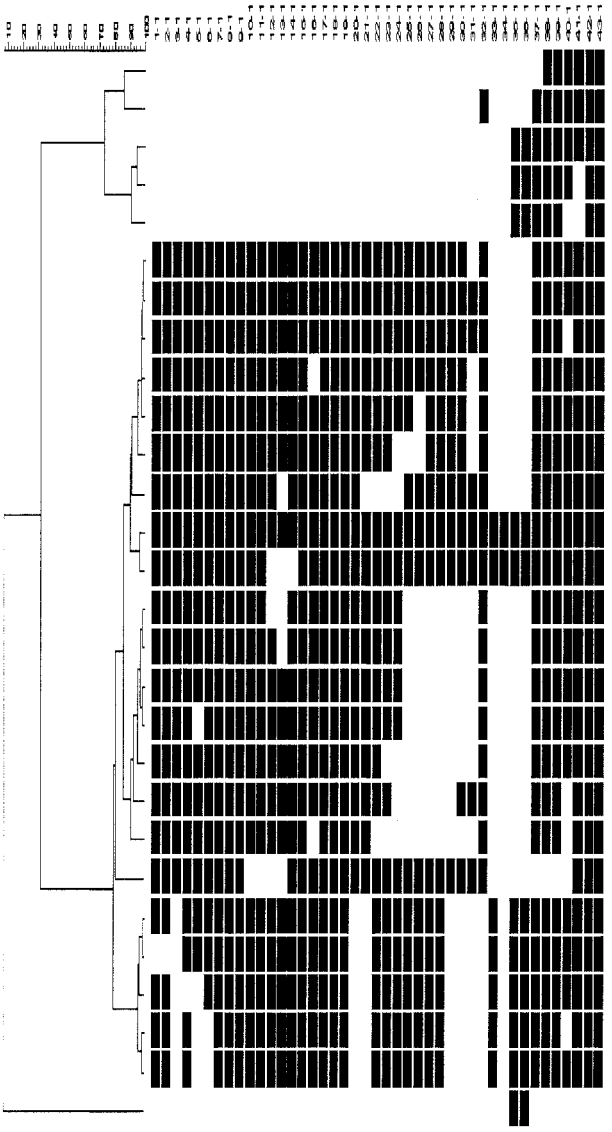


FIG. 1. Dendrogram presentation of 113 spoligotypes detected among 421 *M. tuberculosis* complex isolates collected from four geographic regions of Taiwan in 2002.

alence and spread of the Beijing family genotypes of *M. tuberculosis* among people in different locations (14, 15). The prevalences of the Beijing family genotype strains have been below 2% in South America; 3 to 5% in Central America, Europe, and Africa; 10% in the Middle East; 13% in Oceania; 16% in North America; and as high as 45 to 86% in the Pacific Asian countries (2, 3, 15, 34). Beijing family genotype strains made up 44.4% of the isolates that we examined in this study. Our findings are comparable to those of other prevalence studies performed in South Korea (43%) (34) and Russia (44.5%) (32). They are, however, lower than the figures reported for Vietnam (54%) (2), Hong Kong (70%) (9), and mainland China (86%) (15).

Taiwan is a densely populated island with an area of merely



Cluster No	No of strain	%
1	5	1.2
2	3	0.7
3	172	40.9
4	2	0.5
5	2	0.5
6	21	5.0
7	2	0.5
8	2	0.5
9	16	3.8
10	19	4.5
11	2	0.5
12	3	0.7
13	2	0.5
14	3	0.7
15	3	0.7
16	2	0.5
17	9	2.1
18	6	1.4
19	6	1.4
20	4	1.0
21	2	0.5
22	2	0.5
23	36	8.6
24	2	0.5
25	3	0.7
26	2	0.5
27	2	0.5
28	2	0.5

FIG. 2. Twenty-eight major clustering groups derived from spoligotyping data for 421 *M. tuberculosis* complex isolates collected in 2002.

36,000 km^2. Despite this density and land area, the Beijing family genotype strains of *M. tuberculosis* have a higher prevalence in the northern and eastern regions than in the central and the southern regions of Taiwan. In 2001, the overall tuberculosis notification rate was higher in the eastern region (125.0/100,000 population) than in the southern (70.2/100,000 population), central (66.6/100,000 population), and northern (56.6/100,000 population) regions (10). In addition, a population distribution and density survey conducted by the Ministry of the Interior in 2002 noted a much higher population density in the northern Taiwan region (Taipei metropolitan area, 9,690 people per km^2) than in the eastern Taiwan region (Hualien County, 76 people per km^2) (12). Interestingly, for the isolates that we collected, we found a lower notification rate of tuberculosis caused by Beijing family genotypes among those in highly populated areas and a higher notification rate of tuberculosis caused by Beijing family genotypes among those in relatively less populated areas. Further investigation is needed to determine better how Beijing family strains are disseminated and to shed light on the significant difference in the prevalences of Beijing family genotype *M. tuberculosis* strains in the eastern and the other regions.

It was also interesting that the ancestral strain made up 40% (30 of 75) of isolates of Beijing family genotype strains collected in the southern region, which has a lower prevalence of such strains. In a recent study by Brosch et al. (7), who used an evolutionary scenario for *M. tuberculosis* complex and the presence or absence of an *M. tuberculosis*-specific deletion (TbD1),

TABLE 2. Prevalence of antituberculosis drug resistance among 353 isolates in Taiwan, 2002, by genotype

Genotype	% Resistance to[a]:					
	Any drug	Isonazid	Rifampin	Ethambutol	Streptomycin	MDR[b]
Beijing (n = 181)	46.4	33.7	21.0	27.1	21.0	19.3
Non-Beijing (n = 172)	34.3	24.4	15.7	16.9	19.8	15.7
Odds ratio	1.7	1.6	1.4	1.8	1.1	1.3
95% CI	1.1–2.6	1.0–2.6	0.8–2.6	1.1–3.2	0.6–1.9	0.7–2.3

[a] Isolates resistant to any drug (n = 143; 40.5%), isonazid (n = 103; 29.2%), rifampin (n = 65; 18.4%), streptomycin (n = 72; 20.4%), ethambutol (n = 78; 22.1%), or multiple drugs (MDR) (n = 62; 17.6%).
[b] MDR, multidrug resistant, i.e., isolates resistant to at least isonazid and rifampin.

M. tuberculosis strains were classified into two broad categories: ancestral strains and modern strains. Beijing, Harlem, and African genotype strains have been reported to be modern strains (18). In this study, a total of 44.4% (187 of 421) of the isolates we tested were modern Beijing genotype strains; 13.3% (56 of 421) were ancestral strains. The ancestral strains might have originated from foci of areas where tuberculosis is endemic, as suggested by previous studies (3, 7), and they have probably been spreading continuously in southern Taiwan for a considerable period of time.

The fact that a higher proportion of the Beijing genotype family strains were detected among elderly individuals (46.8%) than among the individuals in the middle-age group (34.0%) can probably exclude the possibility that the Beijing family strains are newly emerging ones just recently introduced into Taiwan. The Beijing family strains have been disseminated in Taiwan for a long time. Also, the fact that a very high proportion of Beijing family genotype strains were detected in individuals below 24 years of age indicates that the Beijing family strains remain dominant and the major causes of recent transmission of tuberculosis in the community.

In this study, the prevalence of antituberculosis drug resistance was higher among the Beijing genotype strains than that among the non-Beijing genotype strains, as has been found in previous studies (5, 13, 25, 33). Nevertheless, since this was a cross-sectional study focused on a set of samples that was probably less than representative, and especially given the limited information about the treatment histories of the patients, it is difficult to draw any concrete conclusions on the relationship between Beijing family genotype strains and the development of resistance to all first-line antituberculosis drugs.

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